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TNFα BLOCKADE IN RHEUMATOID ARTHRITIS: RATIONALE, CLINICAL OUTCOMES AND MECHANISMS OF ACTION

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Abstract — Tumor necrosis factor α (TNFα) is a cytokine with many biological functions of relevance to inflammatory disease. Although only one of several inflammatory mediators produced in abundance in rheumatoid arthritis (RA), experimental data suggest that it is in a dominant position within a cytokine hierarchy and is therefore a prime target for directed immunotherapy in this disease. We have targeted TNFα *in vivo* using a chimerised monoclonal anti-TNFα antibody and have now demonstrated beneficial responses to treatment in three different clinical trials. The results confirm that TNFα is of central importance in the inflammatory process in RA and define a new treatment strategy in this disease.

Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease, characterised by synovial hypertrophy and infiltration by inflammatory cells, progressive erosion of bone and cartilage with regional bony decalcification and systemic features including a marked acute phase response and the production of autoantibodies. The disease is common, with a lifetime prevalence of 1–2% in the U.S., and results in significant disability and economic loss. It has been shown that patients with severe RA have 5 year mortality rates similar to three vessel coronary artery disease or stage IV Hodgkin's disease (Pincus & Callahan, 1986).

Current drug therapy for RA includes the use of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease modifying antirheumatic drugs (DMARDs), which include gold salts, D-penicillamine, methotrexate, azathioprine, hydroxychloroquine, and sulphasalazine. Many patients remain refractory to such conventional treatments or are unable to tolerate them in the long term because of side-effects (Brooks, 1993). In addition, the evidence that most of these therapies alter the progression of disease is poor, and new treatments are urgently needed.

Over the past few years much progress has been made in our understanding of the pathogenesis of autoimmune diseases such as RA. The current paradigm suggests that disease promoting CD4 T-cells become activated following interaction with antigen presenting cells bearing MHC class II structures (Harris, 1990). The activated CD4 T-cells in turn activate other cells including macrophages, B-cells, other T-cells, and synoviocytes to release cytokines, which in turn leads to an inflammatory cascade involving the production of pro-inflammatory cytokines, degradative enzymes and products of lipid metabolism. This leads to synovial inflammation and proliferation with the formation of pannus and destructive joint lesions.

IMPORTANCE OF TNFα IN RHEUMATOID ARTHRITIS

Many cytokines are detectable in synovial fluid in RA, with those present in most abundance being of macrophage and/or fibroblast origin. They include IL-1, IL-6, IL-8, tumor necrosis factor α (TNFα), macrophage colony simulating factor (M-CSF) and granulocyte-macrophage CSF. These factors are pleiotropic in nature and exhibit a high degree of redundancy. Thus, for example, IL-1 and TNF share the capacity to stimulate both chondrocytes and bone cells, thereby potentially contributing to cartilage and bony destruction in RA (reviewed in Feldmann *et al.*, 1993).

Immunoreactive and bioactive TNFα are readily detectable in RA synovial fluid and immunohistochemical studies have confirmed its production within synovial tissue. TNFα is found in the synovial lining layer, the interstitium and in cells at the cartilage/pannus junction (Chu *et al.*, 1991), a site of cartilage destruction in RA. The two receptors for TNFα, p55 and p75, are

expressed in rheumatoid synovium with a similar anatomical distribution to that of TNFα (Deleuran *et al.*, 1992), raising the possibility that TNFα may act in a paracrine fashion, allowing for amplification of inflammatory signals. Soluble TNFα receptors (sTNF-R) are also detectable in synovial fluid, but their concentrations are insufficient to neutralise the high levels of TNFα present, resulting in persistent TNF bioactivity (Cope *et al.*, 1992).

In planning therapeutic interventions involving the targeting of specific inflammatory cytokines, it has been of particular importance to establish how these factors relate to each other. In a series of experiments exploiting an *in vitro* mixed synovial cell culture system, it has been shown that the addition of blocking antibodies to TNFα results in inhibition of IL-1 and GM-CSF secretion, while converse experiments involving the inhibition of IL-1 or GM-CSF function do not reduce TNF production (Brennan *et al.*, 1989; Haworth *et al.*, 1991). These data suggest that although there might be redundancy of cytokines within the synovial compartment in RA, there is also hierarchy, with TNFα in a controlling position.

Apart from its effects on endothelial adhesion molecule expression and on the production of other cytokines, TNFα has a number of other functions which may contribute to joint pathology in RA (reviewed by Vassalli, 1992). Amongst these are the activation of granulocytes and monocytes resulting in enhanced effector function, the stimulation of fibroblast growth and effects on the metabolism of cartilage and bone.

In order to test the hypothesis that TNF was an important regulator of disease *in vivo*, mice with collagen type II-induced arthritis were administered blocking antibodies to TNF either before or after disease onset. In both treatment modes, anti-TNF therapy reduced inflammation and histologic severity in a dose-dependent manner (Williams *et al.*, 1992). In support of these findings, other workers have shown that treatment with TNF in these mice leads to a worsening of disease (Cooper *et al.*, 1992) and that transgenic mouse lines expressing recombinant human TNFα at low levels develop a chronic inflammatory polyarthritis, which is prevented by treatment with anti-TNF antibody (Keffer *et al.*, 1991).

CLINICAL STUDIES WITH ANTI-TNFα

The *in vitro* and murine arthritis experiments provided the basis for our hypothesis: that TNFα is a major molecular regulator in RA, and that blockade of TNFα production or function in man would therefore produce beneficial clinical outcomes (Maini *et al.*, 1993). A variety of therapeutic agents could be used to test this hypothesis *in vivo*, including monoclonal antibodies with specificity for TNFα or for the membrane-bound TNF-R, or sTNF-R themselves. Although each of these approaches has appeal, concerns about possible antibody-induced cell signalling with anti-receptor antibodies and about the possible consequences of development of antibody responses to therapeutically administered sTNF-R led us to choose cA2, an antibody directed to TNFα, as our test agent. cA2 is a chimeric human/mouse monoclonal antibody produced by Centocor Inc., and was constructed from the constant regions of human (h) IgG1κ, together with the Fv region of a neutralising murine anti-hTNFα antibody (A2), as previously described (Knight *et al.*, 1993). The chimeric antibody is of high affinity (K_a 10^{10} M^{-1}), retains specificity for natural and recombinant hTNFα, and is capable of inhibiting a number of TNFα-dependent functions *in vitro*, including endothelial cell E-selectin expression and pro-coagulant activity.

A programme of clinical trials using cA2 in the treatment of patients with RA has now been established, and the results of an open label pilot study published (Elliott *et al.*, 1993). For this trial, we recruited 20 patients who had relatively long-standing and refractory disease. The patients were at the more severe end of the disease spectrum, as judged by number of diseased joints, seropositivity for rheumatoid factor and the presence of erosions. Pre-trial medications including NSAIDs and low-dose corticosteroids were continued at stable doses, but any DMARD therapy was withdrawn at least 4 weeks prior to trial entry. Patients were given cA2 by intravenous infusion as day cases or with an overnight hospital stay, receiving a total of 20 mg/kg antibody in either two or four infusions. Using this dosing regimen, which had been derived by extrapolation from the studies in the collagen II mice, it was possible to detect cA2 in the circulation for several weeks post injection (unpublished data), indicating a prolonged circulating half-time when compared with most non-chimerised antibodies.

The clinical outcome from this study has been reported in detail (Elliott *et al.*, 1993), but in essence, each patient demonstrated a response according to well-accepted composite response measures and the group as a whole showed impressive improvements in swollen joint count, tender joint index, pain score and the other clinical assessments. On average, the clinical improvement lasted 12 weeks, but all patients eventually relapsed.

Although the clinical data were impressive, the changes observed in acute phase measures were even more convincing. Serum C-reactive protein (CRP) and

serum amyloid A (SAA) levels showed marked reductions in most patients, with normalisation in some and highly significant changes in the group as a whole. The erythrocyte sedimentation rate (ESR), a more traditional but complex measure of inflammation in RA, also decreased in many patients. It was clear from these data that TNFα blockade was very effective in controlling the acute phase response, and measurement of serum IL-6 levels suggested that this control was due, at least in part, to regulation of IL-6 production or clearance *in vivo*. It seems likely, however, that changes in the production of other hepatic-active cytokines such as leukaemia inhibitory factor may also be of importance in this phenomenon.

In a second clinical study with cA2, some of the original open label patients have been allowed further infusions of cA2 following relapse in their disease. This time, a dose of only 10 mg/kg cA2 was used, half that administered in the first study, and was repeated up to three times. Although this study is still on-going, it is clear that repeated responses to cA2 are possible and that the magnitude of the responses is maintained with each treatment cycle. The duration of repeated responses has varied in different patients, with average durations of 9 and 8 weeks (treatment cycles 2 and 3, respectively; 10 mg/kg), compared with 12 weeks (cycle 1; 20 mg/kg) (unpublished data).

The data from these studies have been very encouraging and have led to the initiation of a third clinical trial. This is a multi-centre, patient and investigator-blinded, mini-dose ranging study of cA2 versus placebo involving large patient numbers. Preliminary analysis of the results from this study is encouraging, supporting the results of the open label study (unpublished data).

One concern prior to starting these trials was the data from *in vitro* experiments and animal models, suggesting that TNFα was of importance in the control of infection. Thus, short-term exposure of T-cells to TNFα *in vitro* results in enhanced mitogenic responses and its administration *in vivo* confers protection against infection with *Listeria monocytogenes* and some other organisms (Havell, 1989). Mice deficient for the 55 kDa tumor necrosis factor receptor are also more sensitive to *Listeria monocytogenes* infection (Pfeffer *et al.*, 1993). In considering our experience with cA2 in RA, however, there have been no infections with unusual organisms and it is not yet clear whether there is any increase in other, less serious infective events. Interestingly, recent data from our group suggest that although TNFα may be co-mitogenic for T-cells in acute exposure *in vitro*, chronic exposure of T-cell lines to this cytokine results in suppression of antigenic responses (Cope *et al.*, 1994). Such a finding may go some way to provide reassurance about the safety of administration of TNFα-blocking drugs to patients. Other adverse events have also been uncommon and in the short term at least, the infusions themselves have been well tolerated (Elliott *et al.*, 1993).

Since administration of other murine monoclonal antibodies to patients with RA has induced a high frequency of antiglobulin responses (reviewed by Elliott & Maini, 1994), we were particularly interested in the immunogenicity of the chimerised antibody used in these experiments. Only one patient developed detectable anti-chimeric responses in the absence of re-treatment and this was of low titre (unpublished data), suggesting that cA2 is not particularly immunogenic. HACAs were eventually detected in half of the patients in the re-treatment programme, but were mostly of low titre and did not prevent further treatment. Whether the development of HACAs in our patients is of any clinical significance is presently unknown.

The demonstration of effective short-term suppression of rheumatoid disease by cA2 raises the question of how this agent might fit into the therapeutic armamentarium. It appears to be a more powerful anti-inflammatory drug than the NSAIDs, although a direct comparison has not yet been made. The data certainly suggest that cA2 will be a useful agent for the control of acute disease flares, or in the control of disease while awaiting onset of action of DMARDs and in these settings its use may be an attractive alternative to high-dose corticosteroids or cytotoxic therapy. Whether regular use of cA2 as a single agent may allow disease modification has not yet been tested and may depend on our ability to control antiglobulin responses. Strategies which could be adopted to control antiglobulin responses *in vivo* include combination therapy with traditional immunosuppressive drugs, or the co-administration of specific, T-cell directed monoclonal antibodies with the aim of inducing peripheral tolerance (Elliott & Maini, 1994). In recent experiments in our laboratory, anti-TNF and anti-CD4 synergised in the control of murine collagen II arthritis, and antiglobulin responses to the TNF blocking antibody were reduced (Williams *et al.*, 1994). The application of such combination immunotherapy in humans will be of great interest.

MECHANISM OF ACTION OF cA2 IN RA

There are several possible mechanisms by which cA2 might achieve beneficial clinical responses in RA. Most likely is a direct neutralisation of TNFα at the point of

production (the inflamed joint) with clearance of antibody–cytokine complexes by cells of the reticulo-endothelial system. Neutralisation of TNFα action might then result in secondary changes, such as the control of other cytokine expression. The falls in serum IL-6, CRP and SAA in the open label patients are the most visible expression of this mechanism. The extent to which cA2 penetrates joint tissue is not yet clear, however, and actions of cA2 within the vasculature or at the level of the endothelium may also be of importance. Preliminary data suggest that infiltration of inflammatory cells into joint tissue and fluid may be reduced after treatment with cA2, suggesting that TNF blockade reduces cell migration. This might be achieved by deactivation of endothelium, with consequent downregulation of adhesion molecule expression.

CONCLUSIONS

The treatment of RA is entering an exciting phase, with the development of new biological therapies based on an appreciation of the pathogenesis of disease. Our studies demonstrate that TNFα is particularly promising as a therapeutic target and that anti-TNFα is effective both in the short-term suppression of disease and in the setting of repeated use to control disease flares. Long-term use of anti-TNF in RA will provide a challenge, but if successful, may herald a new era in the therapy of this disease.

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